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Effect of Autograft CD34+ Dose on Outcome in Pediatric Patients Undergoing Autologous Hematopoietic Stem Cell Transplant for Central Nervous System Tumors

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Abstract

Background: Consolidation with autologous hematopoietic stem cell transplantation (HSCT) has improved survival for patients with central nervous system tumors (CNSTs). The impact of the autologous graft CD34+ dose on patient outcomes is unknown.

Objectives: To analyze the relationship between CD34^{+} dose, total nucleated cell (TNC) dose, and clinical outcomes, including overall survival (OS), progression free survival (PFS), relapse,

non-relapse mortality (NRM), endothelial-injury complications (EIC), and time to neutrophil engraftment in children undergoing autologous HSCT for CNSTs.

Study Design: A retrospective analysis of the CIBMTR database was performed. Children aged <10 years who underwent autologous HSCT between 2008-2018 for an indication of CNST were included. An optimal cut point was identified for patient age, CD34$^+$ cell dose, and TNC, using the maximum likelihood method and PFS as an endpoint. Univariable analysis for PFS, OS, and relapse was described using the Kaplan-Meier estimator. Cox models were fitted for PFS and OS outcomes. Cause-specific hazards models were fitted for relapse and NRM.

Results: One hundred fifteen patients met the inclusion criteria. A statistically significant association was identified between autograft CD34+ content and clinical outcomes. Children receiving >3.6x10^6/kg CD34$^+$ cells experienced superior PFS (p=0.04) and OS (p=0.04) compared to children receiving 3.6x10^6/kg. Relapse rates were lower in patients receiving >3.6x10^6/kg CD34$^+$ cells (p=0.05). Higher CD34$^+$ doses were not associated with increased NRM (p=0.59). Stratification of CD34$^+$ dose by quartile did not reveal any statistically significant differences between quartiles for 3-year PFS (p=0.66), OS (p=0.29), risk of relapse (p=0.57), or EIC (p=0.87). There were no significant differences in patient outcomes based on TNC, and those receiving a TNC >4.4x10^8/kg did not experience superior PFS (p=0.26), superior OS (p=0.14), reduced risk of relapse (p=0.37), or reduced NRM (p=0.25). Children with medulloblastoma had superior PFS (p<0.001), OS (p=0.01), and relapse rates (p=0.001) compared to those with other CNS tumor types. Median time to neutrophil engraftment was 10 days vs 12 days in the highest and lowest infused CD34+ quartiles, respectively.

Conclusions: For children undergoing autologous HSCT for CNSTs, increasing CD34$^+$ cell dose was associated with significantly improved OS and PFS, and lower relapse rates, without increased NRM or EICs.

INTRODUCTION

Central nervous system tumors (CNSTs) are one of the most-common indications for autologous hematopoietic stem cell transplantation (HSCT) in children.[1] Although many CNSTs are radiation-sensitive, radiotherapy may result in severe neuro-developmental sequelae and/or secondary malignant neoplasms, with younger age at the time of irradiation being the greatest risk factor for these late effects.[2–4] Historically, radiation-sparing treatment strategies resulted in dismal outcomes, with 2-3 year progression free survival (PFS) of 0-34%, and median time-to-relapse of 6-9 months.[5–10] Subsequent studies of children with relapsed/recurrent CNSTs were able to achieve event free survival (EFS) rates approaching 50% via single autologous HSCTs,[11–14] with this approach therefore being integrated into front-line clinical trials for newly diagnosed children, with continual improvements in patient outcomes across tumor types have been achieved via the use of consolidative autologous HSCTs.[15–22] Subsequent trials involving multiple sequential (e.g.

tandem) have shown this to be a relatively safe and effective approach,[23] with additional studies ongoing.[24,25]

The impact of the infused autologous graft (autograft) on patient outcomes for children with CNSTs has not been well described to date. Data from adult patients with a range of solid tumor types has suggested that infusion of higher CD34^{+} doses results in shortened time to neutrophil engraftment, lessened need for supportive care,[26,27] and improved survival.[28,29] However, autologous HSCTs are generally not performed for adults with CNSTs.[30] In addition, pediatric peripheral blood stem cell (PBSC) collections may contain two-to-ten times higher levels of CD34^{+} cells than those of adults, potentially greatly exceeding the level necessary for hematopoietic recovery.[31–36] Selecting the optimal CD34+ cell dose for infusion would help guide the management of patients undergoing autologous transplant for CNSTs.

Prior data has shown an association between higher CD34+ doses and a shortened time to hematopoietic recovery.[26,27] However, more rapid hematopoietic recovery may be associated with a heightened incidence of endothelial-injury related complications (EICs).[37–39] Such complications, including engraftment syndrome (ES), idiopathic pneumonia syndrome (IPS), transplant-associated thrombotic microangiopathy (TA-TMA), and veno-occlusive disease / sinusoidal obstructive syndrome (VOD/SOS) occur due interactions between injured endothelial cells and activated immune effector cells,[40,41] and are a major contributant to non-relapse mortality (NRM).[41,42] Large CD34+ doses could theoretically either improve or worsen outcomes, and the ideal CD34+ autograft content is not known for CNST. This analysis therefore assesses the relationship between CD34^{+} dose and clinical outcomes including progression-free survival, overall survival, relapse rates, non-relapse mortality, endothelial injury complications, and neutrophil engraftment in 115 children who underwent autologous transplant for central nervous system tumors.

METHODS

Data Source

All data were obtained via the Center for International Blood and Marrow Transplant Research (CIBMTR) registry, which contains information on over 575 000 individual patients, obtained from >350 distinct transplant centers world-wide. All data are de-identified and reported on standardized forms, with participation being voluntary, and bias minimized via consecutive reporting requirements. Patients were eligible for inclusion if they underwent an autologous HSCT (either single or tandem) for CNST and were aged under 10 years of age at the time of transplant. Additional inclusion criteria included: transplant occurring in Canada or the United States of America, between the years of 2008-2018 (inclusive). Patients were included if pre-transplant disease status was reported as complete response (CR) or partial response (PR). Children receiving salvage HSCT after failure of primary therapy could not specifically be excluded, but the requirement of a pre-transplant disease status of CR or PR likely minimized the number of included patients with refractory disease. This study was approved by the National Marrow Donor Program's Institutional Review Board.

Patients

Children received treatment according to relevant cooperative group protocols, including Children's Cancer Group (CCG), Pediatric Oncology Group (POG), Children's Oncology Group (COG), National Experimental Therapeutics (NEXT) Consortium, or St. Jude Children's Research Hospital protocols. The study enrollment/treatment protocol was not specifically recorded. Patients undergoing tandem transplants were assessed on the basis of the CD34$^+$ dose of the initial HSCT. If unavailable, the CD34$^+$ dose of a subsequent HSCT was used, based on the assumption that CD34+ dose would be equal between transplant infusions.

Statistical Analysis

Progression-free survival was the primary study endpoint and was defined as alive and in remission. Secondary endpoints included overall survival, relapse rate, non-relapse mortality, incidence of endothelial injury, and time (in days) to neutrophil engraftment (defined as an absolute neutrophil count (ANC) of 0.5 x 10^9/L for three or more consecutive days). The incidence of endothelial-injury related complications (EICs) was assessed as composite variable, and included the occurrence of engraftment syndrome (ES), idiopathic pneumonia syndrome (IPS), veno-occlusive disease / sinusoidal obstructive syndrome (VOD/SOS), thrombotic microangiopathy (TMA), and diffuse alveolar hemorrhage (DAH). Relapse was considered to be recurrence or progression of primary disease, and non-relapse mortality was considered to be death in the absence of disease reoccurrence or progression. Censure was performed at the time of last contact for surviving patients.

Cumulative incidence estimator with Gray's test was utilized to calculate the incidence of EICs, neutrophil engraftment, relapse, and NRM, to accommodate competing risks. The Kaplan-Meier estimator, which estimates the probability of surviving as a function of time, was used to calculate OS (event defined as death from any cause) and PFS (event defined as relapse or death). A Cox model for PFS and OS and a cause-specific hazards model were constructed, and included the following variables: sex, age at the time of transplant, disease status prior to transplant, single or tandem transplant, infused TNC dose, and the infused dose of CD34$^+$ cells. CD34+ and TNC dose cut-points were determined via the maximum likelihood method for PFS, and analyzed as a binary variable, e.g. over/under the cut-point. Stepwise selection was used to select significant variables, with those that achieve a p-value of 0.05 or less being included in the final model; CD34+ cell dose was included in the final model irrespective of significance level attained. P-values of 0.05 were considered statistically significant. No first-order interactions were observed between CD34$^+$ cell dose and the other variables in the final model. Adjusted survival and cumulative incidence curves were created based on the final regression model.[43,44] All analyses were performed via SAS version 9.4 (Cary, NC).

RESULTS

Patient Characteristics

One hundred and fifteen patients were eligible. Patient demographics and HSCT details (including conditioning regimens and EIC incidence) are displayed in Table 1. Median age at the time of HSCT was 3 years (range <1 to 10 years). Seventy-six (66%) of children had a complete response, with the remaining 39 (34%) having a partial response prior to HSCT. Tandem HSCT was performed in 81 (70%) of patients, and single HSCT in 29 (25%). Data regarding number of transplants was unavailable in 5 (4%) of children. Sixty-five (57%) of children had a diagnosis of medulloblastoma. The median length of follow-up for the study population was 67 months (range 9 – 132 months). All (100%) of included patients utilized autologous peripheral blood stem cells as the graft source.

Effect of CD34+ Cell Dose

Autografts contained a median $CD34^+$ of 4.7×10^6/kg (range $0.5\text{-}66.9 \times 10^6$/kg) (Table 1). The study population was also examined based on CD34+ dose quartiles, with the interquartile range being $2.7\text{-}8.3 \times 10^6$/kg (Table 1). Demographic characteristics were similar across CD34+ dose quartiles.

Optimal CD34+ dose: A $CD34^+$ dose of 3.6×10^6/kg was identified as the optimal cell dose "cut point" to discriminate between the largest differences in outcome (Table 2). Autografts which contained $>3.6 \times 10^6$/kg were associated with significantly superior PFS (HR = 0.55, 95% CI 0.31-0.97, p=0.04; Figure 1), superior OS (HR = 0.49, 95% CI 0.25-0.98, p=0.04; Figure 2), and lower relapse rate (HR = 0.56, 95% CI 0.31-1.01, p=0.05; Figure 3). No association was identified between CD34+ cell dose and NRM (HR = 0.46, 95% CI 0.03-7.48, p=0.59). EIC incidence was not associated with CD34+ dose at 100 days, 6 months, and 1-year post-transplant (p=0.91; Table 3).

Interquartile Difference in Outcome: The association between clinical outcomes and $CD34^+$ cell dose was also analyzed by quartiles (Supplementary Table 1). No quartile was associated with a statistically superior outcome for PFS (p=0.66; Figure 4), OS (p=0.29; Figure 5), or relapse rate (p=0.57; Figure 6) at one- or three-years post-transplant. EIC incidence did not vary significantly according to CD34+ dose quartile at 100-days, 6-months, or 1-year post-transplant (p=0.87; Table 4). Descriptive statistics were calculated for patients who received low (e.g. 2×10^6/kg) (n=18) or high (e.g 10×10^6/kg) (n=20) $CD34^+$ cell doses. Among children receiving 2×10^6/kg CD34+ cells, the 3-year PFS and OS were 55.6% and 77.8%, respectively. Children who received 10×10^6/kg CD34+ cells experienced similar outcomes, with 3-year PFS and OS were 65% and 80%, respectively. Three-year relapse rates were: 8/18 (44.4%) of children who received doses 2×10^6/kg relapsed, versus 7/20 (35%) who received 10×10^6/kg CD34+ cells.

Endothelial Injury Complications: Six of the 115 patients (5%) developed EICs, including 5 who experienced IPS, and 1 who experienced VOD/SOS. Among the 6 patients, 3 received conditioning with carboplatin / cyclophosphamide / vincristine, and 3 received conditioning with carboplatin / thiotepa. The median infused CD34+ cell dose among

children who developed EICs was 4.2×10^6/kg (range 1.36-8.74), and the median infused TNC dose was 3.1×10^8/kg (range 0.54-5.44). From the available data, it was not possible to determine whether EICs arose following the first or subsequent HSCT, or whether EICs occurred more frequently after first versus subsequent HSCTs.

Neutrophil Engraftment: Neutrophil engraftment occurred in 114/115 (99.1%) of children by day 28 post HSCT, with the median time to engraftment being 10-12 days across all CD34+ dose quartiles (Table 5). No specific CD34+ dose was found to predict more rapid neutrophil engraftment. The median time to engraft neutrophils was 12 days (range 11-13 days) in the lowest dose quartile compared to 10 days (range 9-11 days) for the highest quartile. All 17 evaluable patients who received a cell dose of 2×10^6/kg engrafted, with a median time-to-neutrophil recovery of 11 days (range 10-12 days). Nineteen of 20 patients (95%) who received a cell dose of 10×10^6/kg had neutrophil recovery by day 28, the median time to neutrophil engraftment 10 days. Data regarding neutrophil recovery following second HSCTs (when performed) were not available.

Effect of TNC Dose

The median TNC was 2.4×10^8/kg (range $0.4-49.8 \times 10^8$/kg), with an interquartile range of $1.3-5.7 \times 10^8$/kg (Table 1). A TNC dose of 4.4×10^8/kg was identified as the optimal cell dose "cut point" to discriminate between the largest differences in outcome. However, there was no significant difference in patient outcomes based on TNC, as patients who received autografts containing a TNC content of $>4.4 \times 10^8$/kg did not have significantly different outcomes from those patients who received autografts containing 4.4×10^8 TNC/kg. Specifically, a TNC $>4.4 \times 10^8$/kg did not result in superior PFS (p=0.26), superior OS (p=0.14), reduced risk of relapse (p=0.37), or reduced NRM (p=0.25).

Overall Outcomes and Effects of Tumor Type and Tandem Transplant

Patient outcomes including PFS, OS, Relapse, NRM, and EIC incidence are displayed in Table 6. Three-year PFS was 58.6% (95% CI 49.4-67.8%) for the study population, and 3-year OS was 75.5% (95% CI 67.4-83.6%). Children with diagnoses other than medulloblastomas experienced worse outcomes than those with medulloblastomas, including lower PFS (HR = 2.87, 95% CI 1.69-5.15, p<0.001), lower OS (HR = 2.59, 95% CI 1.28-5.25, p=0.01), and a higher risk of relapse (HR = 2.67, 95%CI 1.58-4.82, p=0.001) (Table 2).

We assessed for any difference in outcomes based on the number of transplants performed and identified no statistically significant change in outcome between patients who had undergone single versus tandem transplant. Those who underwent tandem HSCT did not have demonstrable improvements PFS (HR 0.74, 95%C CI 0.39-1.41, p=0.36), OS (HR 1.03, 95% CI 0.46-2.29, p=0.94), or risk of relapse (HR 0.69, 95% CI 0.36-1.34, p=0.27), compared to those who underwent single HSCT (Supplementary Table 2).

DISCUSSION

In a retrospective analysis of 115 children undergoing autologous HSCT for central nervous system tumors, higher CD34$^+$ doses were associated with significantly improved PFS and OS, and a lessened risk of relapse. Specifically, patients who received greater than 3.6x10^6/kg had superior outcomes compared to those receiving lower doses, and the administration of higher CD34$^+$ cell doses did not result in increased NRM or EIC.

This is one of the first studies in pediatric stem cell transplantation to show such associations. In contrast, the impact of the autograft CD34+ cell dose on outcomes has been well described in a number of adult studies, with variable results.[28,29,45] Several adult studies have not shown a correlation between CD34+ dose and outcome,[45] while others have shown an association between higher CD34$^+$ doses and improved PFS and OS.[28,29] Additionally, adult data has suggested that CD34$^+$ doses below 2x10^6/kg may delay hematopoietic recovery,[46,47] while autograft cell doses over 5x10^6/kg CD34$^+$ may be been associated with more rapid engraftment.[26,27] Compared to adult autograft trials, CD34+ cell doses are typically much larger in the pediatric population.[29,45] Thus, pediatric-specific data are therefore needed.

One rationale for conducting our current study was to assess whether higher CD34+ cell doses were associated with an increased NRM and EIC, given the potential interaction between immune effector cells and endothelial cells in the transplant process. [40,41] In our current trial, we did not identify any association between EIC incidence, NRM, and CD34+ dose.

Given that CD34+ cells make up approximately 1-3% of the autologous graft,[48] the impact of TNC content upon HSCT outcomes was also examined in our trial. The majority of cells in a typical autograft are a heterogeneous mixture of lineage-differentiated progenitors and immune effector cells (IECs).[49–51] Although we did not observe differences in outcome based on infused TNC, we lacked the ability to investigate specific sub-mononuclear cell populations within the graft. Future prospective investigations into this area are warranted, however, because the importance of IECs in disease control is being increasingly understood. Promising clinical trials have investigated the use of directly modify T cells and NK cells to facilitate tumor targeting.[52,53] Congruently, there is expanding use of anti-tumor vaccines, immune checkpoint inhibition, and oncolytic viral therapies, all of which rely to some extent on the patient's own immunologic response, as mediated by endogenous unmodified IECs.[52,53] Granular investigations into autologous graft IEC content may therefore yield actionable data as these clinical trials continue to grow in number and efficacy.

The observed OS and PFS compared favorably to other autograft studies in children with CNSTs. CCG 99703 used a triple tandem HSCT approach to treat children with embryonal CNSTs, with a 5-year OS of 63.6 ± 5% and 5-year EFS of 43.9 ± 5.2%.[23] HeadStart I reported 2-year OS and EFS of 55±6% and 39±7% respectively.[15] HeadStart II reported 3-year OS and EFS rates of 60% and 49%,[16–18] with HeadStart III noting 5-year OS and EFS rates of 62 ± 5% and 46 ± 5%, respectively, for patients with

medulloblastoma.[21] In general, patients with medulloblastoma have experienced superior outcomes on these trials, compared to patients with other diagnoses.[16–18] Results for the current clinical trials COG ACNS0334 and HeadStart IV are not yet available. Several features of our study may explain the superior outcomes we observed. First, only patients who underwent auto-HSCT were included, with the effect of excluding patients with the most advanced/aggressive CNSTs. Patients who died, had progressive disease, or were otherwise ineligible for transplant were therefore not represented in our results. Second, the study window encompassed only transplants performed from 2008-2018. The results of the NEXT Consortium's HeadStart I and II clinical trials had been published by early 2008,[17,18] HeadStart III closed to accrual in December 2009, and HeadStart IV opened to accrual in September 2015.[24] The COG's ACNS0334 clinical trial likewise opened to accrual in October 2007, and closed in December 2016.[25] We therefore examined a population of patients who likely benefited from recent progress in CNST treatment, and received therapy according to current multicenter trials, as 70% of participants in our study received tandem HSCTs. Lastly, patients >10 years of age were excluded, due to differences in disease biology, management strategies, and the prognosis in this older population.[54,55] The observed NRM in our study likewise compared favorably to other pediatric CNST transplant studies. The day-100 NRM for the HeadStart I, II, and III trials were 6.4% (n=3), 2.1% (n=1), and 0.8% (n=1), respectively.[5] CCG-99703 reported a "toxic mortality rate" of 2.5% (n=2), attributed to the auto-HSCTs performed on that study.[23]

No difference in outcomes was seen based upon whether patients received single versus tandem transplants. However, existing studies would suggest the superiority of tandem transplant in the CNS tumor setting.[23–25] Although this may therefore be a surprising observation, the goal of this investigation was not to assess for the effect of transplant number. Neither study methodology nor sample-size were optimized for this endpoint. It can therefore only be reported that the observed associations between CD34+ cell dose and patient outcomes was not influenced by the number of transplants performed.

Our study has a number of limitations. First, we were necessarily limited to data fields included by the CIBMTR, which do not capture the induction regimen used, number of cycles of pre-apheresis or pre-transplant chemotherapy, and presence/absence of marrow disease at the time of diagnosis, transplant, or apheresis. We are therefore unable to assess the impact of specific treatment protocols on outcomes. We were also unable to analyze for an association between CD34+ dose and platelet recovery or hospitalization length, as this data was not captured.[56,57] Second, our findings may have been confounded by pre-transplant disease burden or treatment response, as this data was not specifically captured. Patients with greater disease burden may have been less able to mobilize CD34+ cells, and therefore both collected and received lower CD34+ doses. Conversely, patients receiving higher CD34+ doses may have been those who disease was less severe. This effect has been seen in adult patients with lymphoma,[58,59] but it is unclear whether it applies to children with CNSTs. We sought to minimize the influence of this by including only patients who had partial or complete responses to therapy, but patient disease status may still have influenced our observed results. We also assessed the cohort of patients who received CD34$^+$ cell doses 2.0x10^6/kg. Although the small size of this group precluded valid statistical modelling, these children did not have notably poorer outcomes based on

descriptive statistics alone. Third, our population was heterogeneous, and included patients with a variety of different CNSTs. The observed effect of CD34$^+$ dose was independent of diagnosis, but disease-specific subset analysis may have resulted in different outcomes for the different types of CNSTs. Due to the relatively small number of patients with any specific, non-medulloblastoma diagnosis, we did not perform such an analysis.

In conclusion, we identified an association between higher autograft CD34$^+$ content and superior PFS and OS, and lower relapse rates. There was no association between CD34+ dose and the occurrence of NRM or EIC. The effect of CD34+ cell dose upon patient outcomes suggests that there may be sufficient grounds to investigate whether a higher CD34$^+$ target might yield improved outcomes in pediatric patients with CNSTs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflicts of Interest and Financial Disclosure Statement

No funding was secured specifically for this project.

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Dr. Wall reports steering committee participation for CRISPR/Vertex Pharmaceuticals and Editas Medicine; acting as a study advisor for CRISPR/Vertex Pharmaceuticals and Editas Medicine; acting as a clinical trial site-PI for CRISPR Therapeutics, Vertex Pharmaceuticals, and Novartis; and research funding from CRISPR Therapeutics, Vertex Pharmaceuticals, and Novartis.

Dr. Rotz reports acting as a resource for Clinical Investigation in Blood and Marrow Transplantation (RCI BMT) (employment).

Dr. Rangarajan reports serving as the BMT Medical Monitor for NMDP (no financial reimbursements); and having served as honorary consultant for Medexus.

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Highlights

- CD34+ doses $>3.6 \times 10^6$/kg were associated with higher progression free and overall survival.

- No association between CD34+ cell doses and post-transplant complications.

- No association between TNC and survival, relapse risk, or non-relapse mortality.
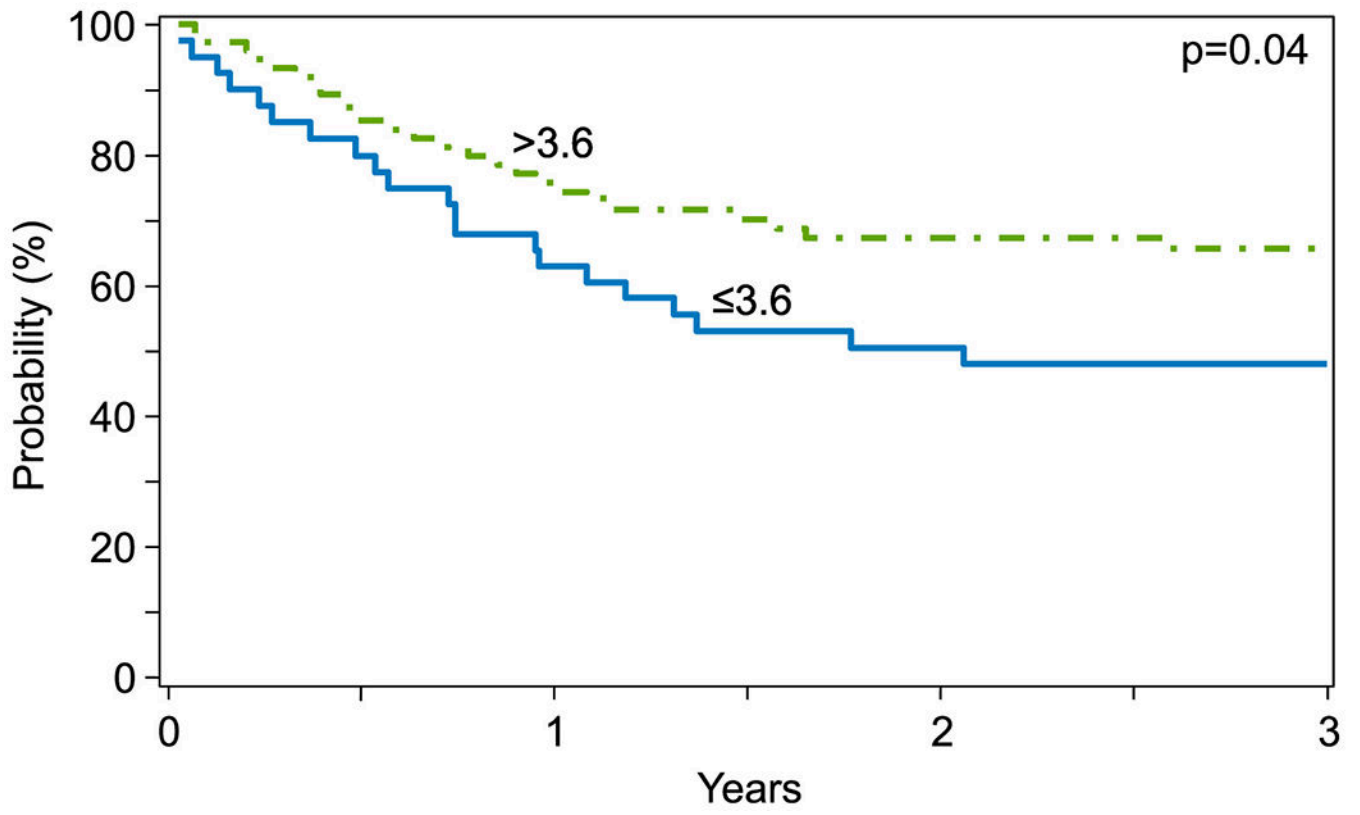
Figure 1:
Progression-free survival by CD34^{+} dose (x10^6/kg)
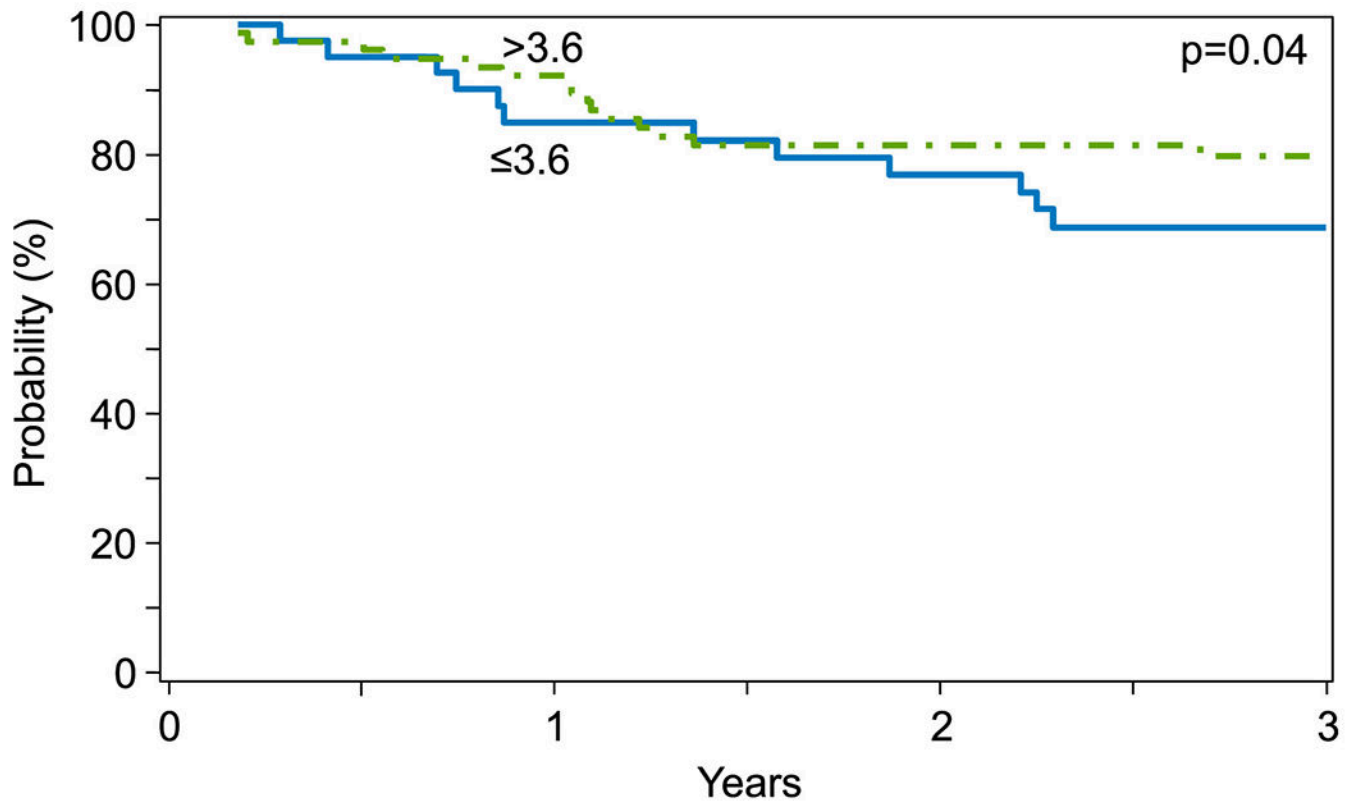
Figure 2:
Overall survival by CD34$^+$ dose (x10^6/kg)

Figure 3:
Relapse rate by CD34$^+$ dose (x10^6/kg)
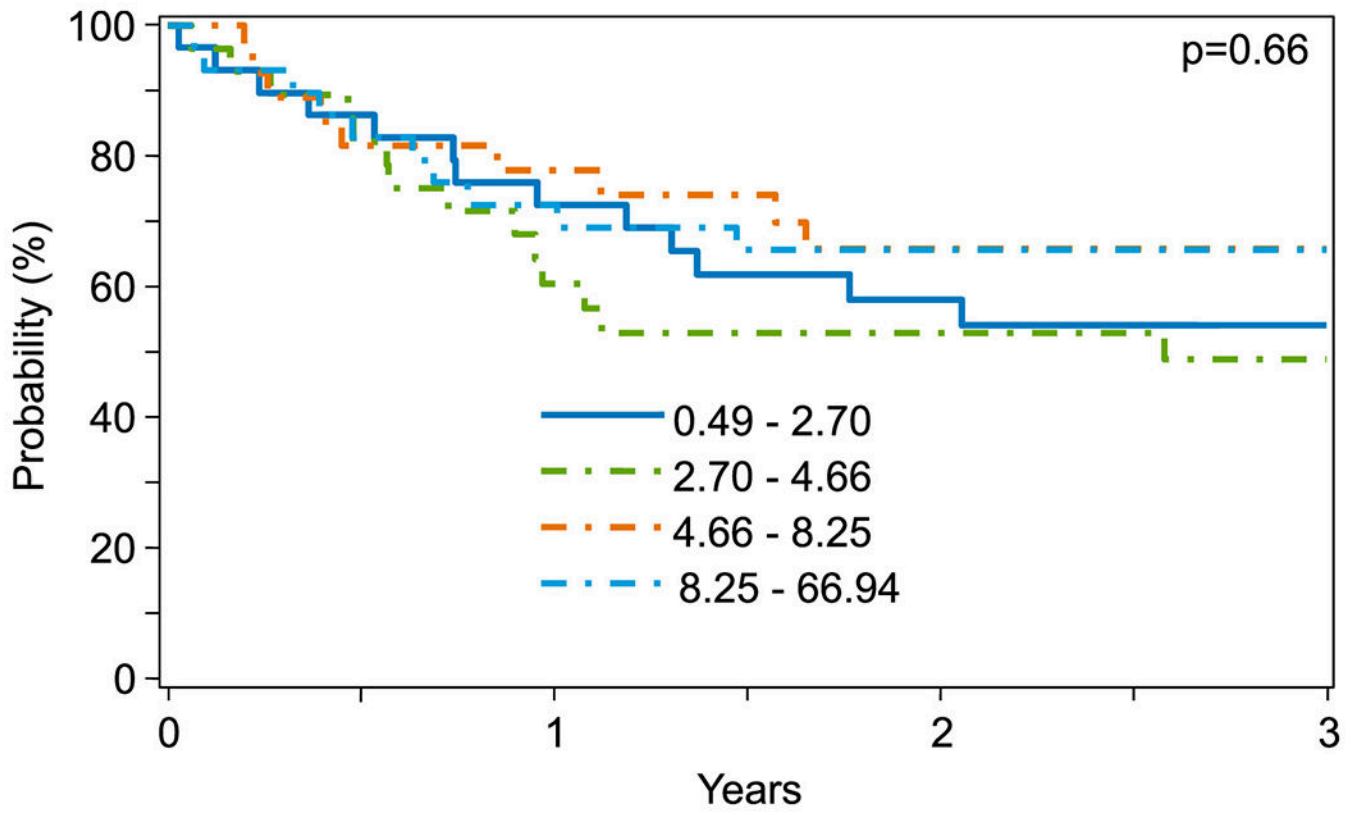
Figure 4:
Progression-free survival by CD34^{+} dose quartile (x10^{6}/kg)

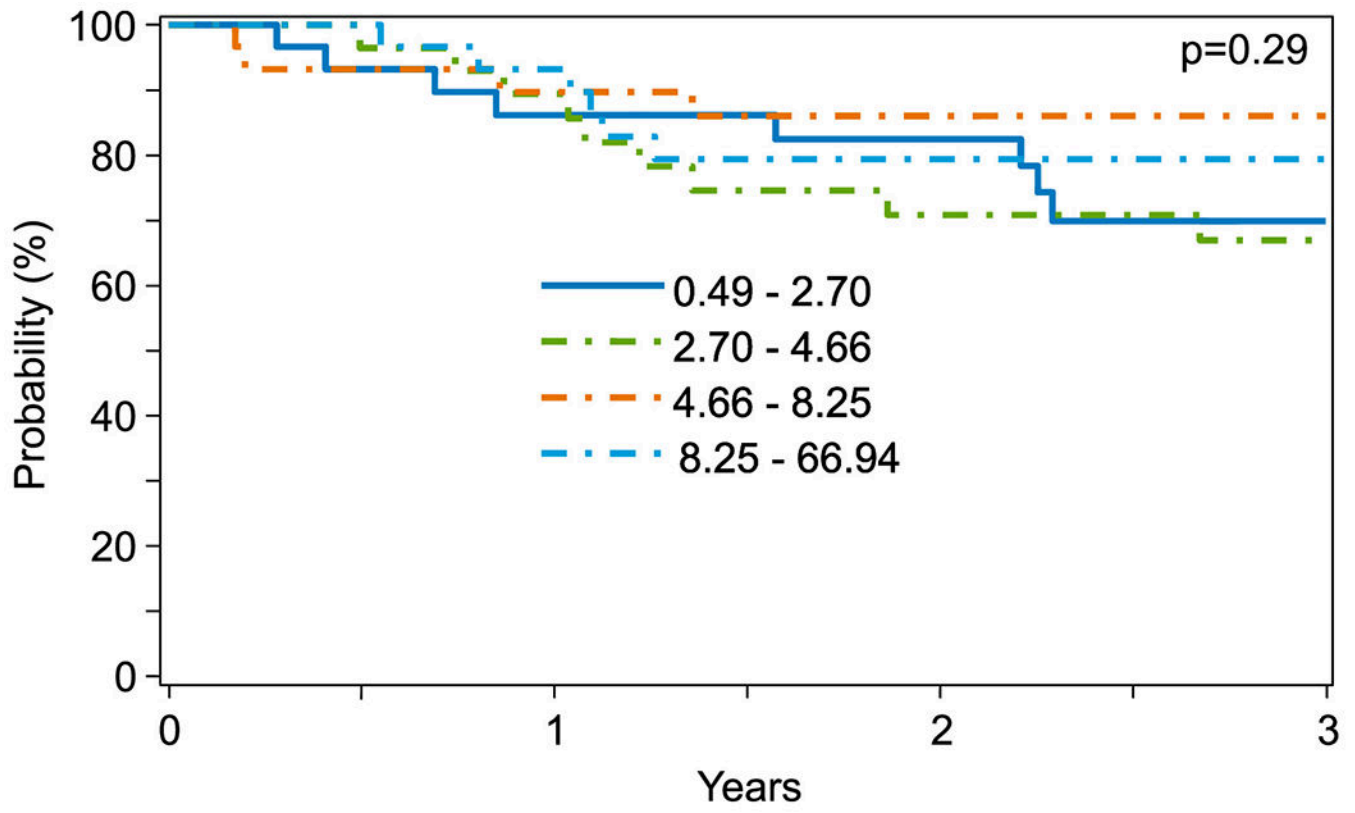**Figure 5:**
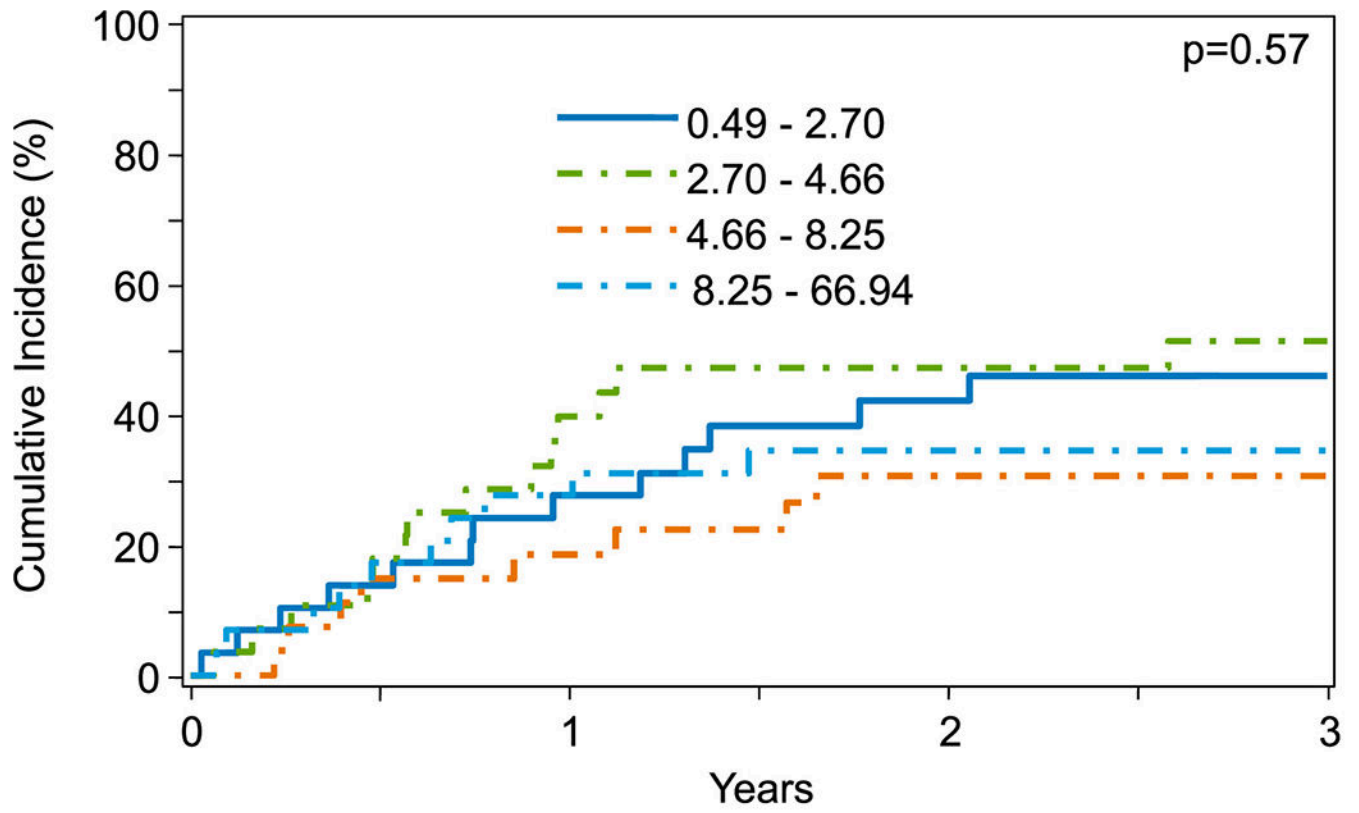Overall survival by CD34$^+$ dose quartile (x10^6/kg)

Figure 6:
Relapse rate by CD34+ dose quartile (x10^6/kg)

Table 1.

Characteristics of patients undergoing autologous HSCT for Central Nervous System Tumors

	Study Population	CD34+ Dose by Quartile (x10⁶/kg)			
		0.49 - 2.70	2.71 - 4.66	4.67 - 8.25	8.26 - 66.94
No. of patients (%)	115 (100)	29 (25.2)	28 (24.3)	29 (25.2)	29 (25.2)
Age at transplant, years - no. (%)					
Median (min-max)	3 (1-10)	5 (1-9)	5 (1-10)	2 (1-8)	3 (1-7)
Less than 1	6 (5)	1 (3)	1 (4)	3 (10)	1 (3)
1 to 2	42 (37)	8 (28)	4 (14)	13 (45)	17 (59)
3 to 5	40 (35)	8 (28)	12 (43)	10 (34)	10 (34)
6 to 9	27 (23)	12 (41)	11 (39)	3 (10)	1 (3)
Sex - no. (%)					
Male	74 (64)	21 (72)	13 (46)	23 (79)	17 (59)
Female	41 (36)	8 (28)	15 (54)	6 (21)	12 (41)
CNS tumor type – no. (%)					
Medulloblastoma	65 (57)	16 (55)	20 (71)	15 (52)	14 (48)
Other CNS tumors [*]	50 (43)	13 (45)	8 (29)	14 (48)	15 (52)
Disease status prior to transplant - no. (%)					
Complete response	59 (51)	11 (38)	17 (61)	13 (45)	18 (62)
Complete response - undetermined	17 (15)	7 (24)	2 (7)	7 (24)	1 (3)
Partial response	39 (34)	11 (38)	9 (32)	9 (31)	10 (34)
Transplant type - no. (%)					
Single HSCT	29 (25)	4 (14)	10 (36)	6 (21)	9 (31)
Tandem HSCT	81 (70)	24 (83)	18 (64)	21 (72)	18 (62)
Not reported	5 (4)	1 (3)	0 (0)	2 (7)	2 (7)
CD34+ dose, cells x10⁶/kg					
Median (min-max)	4.7 (0.5 – 66.9)	1.7 (0.5-27)	37 (2.8-4.5)	6.0 (4.7-8.1)	12.1 (8.3-66.9)
Inter-quartile range	2.7 – 8.3	0.9-2.4	3.2-4.0	5.0-6.7	9.6-17.5
Total nucleated cell dose, cells x10⁸/kg					
Median (min-max)	2.4 (0.4 – 49.8)	3.8 (0.4-48.4)	5.1 (0.8-47.6)	1.4 (0.4-49.8)	2.2 (0.8-13.4)
Inter-quartile range	1.3 – 5.7	2.2-6.5	1.9-9.7	0.9-3.2	1.4-5.5
Follow-up, months					
Median (min-max)	67 (9-132)	54 (9-124)	73 (11-132)	74 (12-144)	76 (23-124)
Conditioning Regimen of First Transplant– no. (%)					
Cyclophosphamide / Fludarabine	1 (1)				
Carboplatin / Etoposide	27 (23)				
Carboplatin / Thiotepa	59 (51)				
Carboplatin	3 (3)				

	Study Population	CD34+ Dose by Quartile (x10⁶/kg)			
		0.49 - 2.70	2.71 - 4.66	4.67 - 8.25	8.26 - 66.94
Carboplatin / Cyclophosphamide / Vincristine +/− Amifostine	23 (20)				
Etoposide / Thiotepa	1 (1)				
None	1 (1)				

[*] Other CNS tumors: Rhabdoid tumors (n=14), Pineoblastoma (n=10), Anaplastic astrocytoma (n=3), Cerebral neuroblastoma (n=2), Anaplastic ependymoma (n=1), Ependymoblastoma (n=1), Glioblastoma multiforme (n=1), Primary brain sarcomas (n=1), Other primitive neuroectodermal tumors (n=13), Other high grade glial tumors (n=2), Not reported (n=1).

Table 2.

Multivariable Analysis – Outcomes

Progression-Free Survival					
	N	**HR**	**95% CI Lower Limit**	**95% CI Upper Limit**	**p-value**
CD34+dose (x10^6/kg)					
3.6	42	1			
>3.6	71 [*]	0.55	0.31	0.97	0.04
CNS type					
Medulloblastoma	64	1			
Other CNS tumors	49	2.87	1.60	5.15	<0.001

Overall Survival					
	N	**HR**	**95% CI Lower Limit**	**95% CI Upper Limit**	**p-value**
CD34+dose (x10^6/kg)					
3.6	42	1			
>3.6	73	0.49	0.25	0.98	0.04
CNS type					
Medulloblastoma	65	1			
Other CNS tumors	50	2.59	1.28	5.25	0.01

Relapse					
	N	**HR**	**95% CI Lower Limit**	**95% CI Upper Limit**	**p-value**
CD34+dose (x10^6/kg)					
3.6	42	1			
>3.6	71 [*]	0.56	0.31	1.01	0.05
CNS type					
Medulloblastoma	64	1			
Other CNS tumors	49	2.67	1.48	4.82	0.001

Non-Relapse Mortality					
	N	**HR**	**95% CI Lower Limit**	**95% CI Upper Limit**	**p-value**
CD34+dose (x10^6/kg)					
3.6	42	1			
>3.6	71 [*]	0.46	0.03	7.48	0.59

[*] Progression-free survival, relapse, and non-relapse mortality data were not available for 2 patients.

Table 3.

EIC Incidence by Optimal CD34$^+$ Dose

	3.6 x10^6/kg (N=42)	>3.6 x10^6/kg (N=71)[*]	p= 0.91
100-day	0%	4.1% (95% CI = 0.8-9.9%)	
6 months	2.5% (95% CI = 0-9.6%)	4.1% (95% CI = 0.8-9.9%)	
1-year	5% (95% CI = 0.5-13.9%)	4.1% (95% CI = 0.8-9.9%)	

[*] Data unavailable for 2 patients

Table 4.

EIC Incidence by CD34+ Dose Quartiles

	CD34+ Dose (x10^{6}kg)				p-value
	0.49-2.70 (N=28)	2.71-4.66 (N=28)	4.67-8.25 (N=29)	8.26-66.94 (N=28)	
EIC Incidence	Probability of EIC (95% CI)				
100-day	0%	0%	6.9% (0.6-19)	3.6% (0-13.7)	0.87
6 months	3.6% (0-13.7)	0%	6.9% (0.6-19)	3.6% (0-13.7)	
1-year	7.1% (0.7-19.7)	0%	6.9% (0.6-19)	3.6% (0-13.7)	

Table 5.

Time to Neutrophil Engraftment by CD34+ Dose Quartiles

	CD34+ Dose (Number of Patients)			
	0.49–2.70 x10^6/kg (N=28)	2.71–4.66 x10^6/kg (N=28)	4.67–8.25 x10^6/kg (N=29)	8.26–66.94 x10^6/kg (N=28)
Days to neutrophil engraftment				
Median (min-max)	12 (1-16)	10 (1-22)	11 (9-19)	10 (9-22)
5th-95th percentile	1-16	4-15	10-13	9-14
25th-75th percentile	11-13	10-12	10-12	9-11

Table 6:

Patient Outcomes

Outcomes	N	CNS Tumors (N = 115)
		% Prob (95% CI)
Progression-free survival	113	
1-year		70.8% (62.1-78.8)
3-year		58.6% (49.4-67.8)
Overall survival	115	
1-year		89.5% (83.3-94.4)
3-year		75.5% (67.4-83.6)
Relapse	113	
1-year		28.4% (20.4-37)
3-year		40.5% (31.3-49.8)
Non-relapse mortality	114	
1-year		0.9% (0-3.5)
3-year		0.9% (0-3.5)
Endothelial Injury Complications	115	
100-day		4.3% (1.4-8.8)
1-year		6.1% (2.5-11.2)

[*] Endothelial-injury complications is a composite endpoint consisting of time to first of the following events: engraftment syndrome, IPS, VOD/SOS, thrombotic microangiopathy, or diffuse alveolar hemorrhage